

TRANSMISSION THRESHOLDS FOR DENGUE IN TERMS OF *Aedes aegypti* PUPAE PER PERSON WITH DISCUSSION OF THEIR UTILITY IN SOURCE REDUCTION EFFORTS

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Abstract. The expense and ineffectiveness of drift-based insecticide aerosols to control dengue epidemics has led to suppression strategies based on eliminating larval breeding sites. With the notable but short-lived exceptions of Cuba and Singapore, these source reduction efforts have met with little documented success; failure has chiefly been attributed to inadequate participation of the communities involved. The present work attempts to estimate transmission thresholds for dengue based on an easily-derived statistic, the standing crop of *Aedes aegypti* pupae per person in the environment. We have developed these thresholds for use in the assessment of risk of transmission and to provide targets for the actual degree of suppression required to prevent or eliminate transmission in source reduction programs. The notion of thresholds is based on 2 concepts: the mass action principal—the course of an epidemic is dependent on the rate of contact between susceptible hosts and infectious vectors, and threshold theory—the introduction of a few infectious individuals into a community of susceptible individuals will not give rise to an outbreak unless the density of vectors exceeds a certain critical level. We use validated transmission models to estimate thresholds as a function of levels of pre-existing antibody levels in human populations, ambient air temperatures, and size and frequency of viral introduction. Threshold levels were estimated to range between about 0.5 and 1.5 *Ae. aegypti* pupae per person for ambient air temperatures of 28°C and initial seroprevalences ranging between 0% to 67%. Surprisingly, the size of the viral introduction used in these studies, ranging between 1 and 12 infectious individuals per year, was not seen to significantly influence the magnitude of the threshold. From a control perspective, these results are not particularly encouraging. The ratio of *Ae. aegypti* pupae to human density has been observed in limited field studies to range between 0.3 and >60 in 25 sites in dengue-endemic or dengue-susceptible areas in the Caribbean, Central America, and Southeast Asia. If, for purposes of illustration, we assume an initial seroprevalence of 33%, the degree of suppression required to essentially eliminate the possibility of summertime transmission in Puerto Rico, Honduras, and Bangkok, Thailand was estimated to range between 10% and 83%; however in Mexico and Trinidad, reductions of >90% would be required. A clearer picture of the actual magnitude of the reductions required to eliminate the threat of transmission is provided by the ratio of the observed standing crop of *Ae. aegypti* pupae per person and the threshold. For example, in a site in Mayaguez, Puerto Rico, the ratio of observed and threshold was 1.7, meaning roughly that about 7 of every 17 breeding containers would have to be eliminated. For Reynosa, Mexico, with a ratio of approximately 10, 9 of every 10 containers would have to be eliminated. For sites in Trinidad with ratios averaging approximately 25, the elimination of 24 of every 25 would be required. With the exceptions of Cuba and Singapore, no published reports of sustained source reduction efforts have achieved anything near these levels of reductions in breeding containers. Practical advice on the use of thresholds is provided for operational control projects.

Today, most dengue control efforts are based on suppression of *Aedes aegypti* (L.) and not eradication; increasingly, these efforts rely on reducing the number of larval breeding habitats and not on insecticides.^{1–3} How should such source reduction efforts be monitored? In terms of risk assessment, what levels of elimination are necessary to preclude transmission? Several investigators have argued that the traditional *Stegomyia* indices, as epidemiologic indicators of dengue transmission, should be abandoned because they have a number of serious shortcomings.^{3,4} The case has recently been made that a pupal and demographic survey, providing an estimate of the number of pupae per person in a community, is more appropriate for assessing risk and directing control operations.⁴ This method uses the ratio of pupae per person for several reasons. 1) Unlike any of the other life stages, it is possible to actually count the absolute number of *Ae. aegypti* pupae in most domestic environments.^{1,2} 2) Container-inhabiting *Stegomyia* pupae are easily and inexpensively separated from other genera and identified to species as emerged adults or pupae. 3) Because pupal mortality is slight and well-characterized, the number of pupae is highly correlated with the number of adults.^{5–7} The obvious need

is to be able to relate this ratio to the risk of transmission, taking into account the role of ambient temperature and herd immunity. The present work describes an attempt to develop transmission thresholds for dengue in terms of these factors. Also considered is the influence of the magnitude of the viral introduction(s) and the importance of the stochastic nature of nascent epidemics. We have provided a discussion on how these can be used in risk assessments and targeted source reduction programs to control dengue.

METHODS

The estimates of transmission thresholds developed here are based on a pair of simulation models (the Container-Inhabiting Mosquito Simulation Model [CIMSIM] and the Dengue Simulation Model [DENSIM]) developed to provide site- and weather-specific insight into the dynamics and control of dengue viruses and their vectors.^{6,8,9} These models reflect a long history of mathematical modeling of epidemiologic phenomenon. As early as 1906, Hamer postulated that the course of an epidemic depended on the rate of contact between susceptible and infectious individuals;¹⁰ this no-

tion, the mass action principal, has become a central concept in mathematical epidemiology—the rate of spread of an infection within a population is proportional to the product of the density of susceptible and infectious people. Ross used this principal in his pioneering work on the dynamics of malaria transmission.¹¹ The insight of Hamer and Ross was further developed by Kermack and McKendrick in 1927 into an understanding of the concept of thresholds.¹² Anderson and May consider this threshold theory, coupled with the mass action principal, to be the cornerstone upon which modern epidemiologic theory is built.¹³ The notion of thresholds indicates that the introduction of a few infectious individuals into a community of susceptible individuals will not give rise to an epidemic outbreak unless the density of susceptible individuals (or vectors) is above a certain critical level. More recent advances in the rapid growth of mathematical epidemiology involve recognition that variation and the elements of chance are important determinants of the spread and persistence of infection and have led to the development of stochastic models.

Citing the abstract nature of much of the theoretical work and the lack of ties to field data, Anderson and May make the observation that little use of this theoretical understanding has been made in empirical studies and the development of public health policy regarding infectious human diseases.¹³ They find that “in view of the successes achieved by combining empirical and theoretical work in the physical sciences, it is surprising that many people still question the potential usefulness of mathematical models in epidemiology”. The models used in this study incorporate the theoretical principals outlined above, but in a computer simulation environment that permits site-specific information to be used.

The models. Our threshold analysis uses two, interrelated simulation models: the CIMSIm and the DENSIm. Descriptions and validation studies of these models have been presented earlier.^{6,8,9} In this analysis, CIMSIm and DENSIm are used to estimate transmission thresholds as a function of pupae per person, herd immunity, ambient temperatures, and the magnitude of the viral introduction(s). Briefly, CIMSIm is used to integrate a host of factors pertaining to vector dynamics and provides the entomological inputs to DENSIm. Whereas CIMSIm is essentially an accounting program of vector dynamics, DENSIm is the corresponding account of human dynamics, keeping track of birth and death, individual serologies, and modeling the dynamics of virus transmission between hosts and vectors. Both models are weather-driven and stochastic.

Definition of epidemics and types of viral introductions. We use a definition of an epidemic that is arbitrary but useful from a public health point of view—any single year where seroprevalence increases by at least 10% is considered to be an epidemic year. Ten percent was selected because any disease involving that proportion of the population would be considered an epidemic and this level of transmission would result in just slightly more than 1% of the population being infected during the peak of the epidemic—a minimum value that has been suggested as sufficient for the detection of transmission.^{6,14} Just how many mosquitoes per person are required to support this level of transmission is a function of many factors, but the ones consid-

ered key determinants are the number, size, and timing of viral introductions during the year, seroprevalence of antibody to dengue, and temperature.⁶ We will develop estimates for scenarios where 1, 2, 4, or 8 viremic person(s) are introduced into an area on day 90 of the year (approximately the end of March), and for the case where a single viremic person is introduced once-a-month throughout the year; for convenience, we will name these Single Introduction_{1, 2, 4, and 8} and Monthly Introductions, respectively. In these assessments we make several important assumptions that are likely to be true in most tropical locations: 1) vector competence is adequate, 2) blood feeding by *Ae. aegypti* occurs primarily (>90%) on humans, and 3) essentially all hosts are at risk of being bitten.⁶ The conditions in the southeastern United States are an obvious exception to these assumptions.

Gonotrophic development rate, adult size, and daily survival. The CIMSIm was used to develop estimates for DENSIm using constant temperatures between 20 and 34°C in 2° steps. To mimic the average daily temperature fluctuation between the daytime high and the nighttime low typically seen in our weather files for tropical locations, the maximum and minimum temperatures used in CIMSIm and DENSIm were plus and minus 5°C of the desired constant temperature. The parameters estimated using CIMSIm were values for gonotrophic development rate, adult size, and survival for each of the temperatures used. We assumed that atmospheric moisture was adequate so as to not limit daily adult (0.89) or egg (0.99) survival.⁸ The representative container types used in CIMSIm to make these estimates were those described by Focks and others for Honduras.⁶

The relationship between standing crops of female adults and pupae. Because temperature influences the duration of pupal development (PD_{temp}), temperature also influences the relationship between the standing crops of pupae and adults. Under cool conditions a smaller proportion of the standing crop of pupae emerge each day than at higher temperatures; specifically, under steady-state conditions, the proportion emerging daily is $1/PD_{temp}$. As a consequence, under cooler conditions, a larger standing crop of pupae will be associated with a given adult population than at warmer conditions. Under equilibrium conditions of constant recruitment and survival, the number of female adult *Ae. aegypti* emerging each day will be the product of the number of pupae present, $1/PD_{temp}$, i.e., that portion of the standing crop of pupae expected to emerge each day, the proportion of pupae that are females (0.50), and the rate of successful emergence (0.83).⁸ The resulting steady-state standing crop of female adults of all ages would be this daily number of emerging females times the expected lifespan of adult ($1/(-\log_e [Sa])$) where Sa is the daily adult survival probability.⁸

Threshold determinations using DENSIm. The DENSIm was parameterized with the previously-published default parameters values, with three exceptions based on unpublished studies. 1) Virus was assumed to titer in the human host at 10^5 mosquito infection doses ($MID_{s_{50}}$). 2) The average number of interrupted feeding attempts per replete blood meal was set to 3.0. 3) The probability that an interrupted feeding attempt was resumed on a different host was 0.35. Each simulation iteration began with an initial human population of 10,000 with an age distribution and age-specific birth and death rates typical of Honduras in the 1980s.⁶

TABLE 1

Daily percentage survival of adults (S_{adult}), eggs (S_{egg}), larvae (S_{larval}), and pupae (S_{pupal}), the possibility of egg hatch, the possibility of an adult population under conditions of constant temperatures, daily gonotrophic development rate ($GD_{\text{rate/da}}$), and wet weight (Weight) of emerging *Aedes aegypti* as indicated by the container-inhabiting mosquito simulation model (CIMSIM)*

Temp. (°C)	Population	S_{adult}	S_{egg}	S_{larval}	S_{pupal}	Egg hatch	$GD_{\text{rate/da}}$	Weight (mg)
20	—	89	96.5	99	99	—	†	†
22	+	89	92	99	99	+	0.165	0.233
24	+	89	88	99	99	+	0.199	0.232
26	+	89	84	99	99	+	0.240	0.233
28	+	89	78–80	99	99	+	0.288	0.236
30	+	89	68–72	99	99	+	0.344	0.244
32	+	89	58–62	99	99	+	0.411	0.275
34	+	89	52–55	99	99	+	†	†
36	—	81	46–49	99	99	+	†	†
38	—	64	39–42	99	99	+	†	†
40	—	47	32–35	88–99	88–99	+	†	†
42	—	30	25–28	53–70	53–70	+	†	†
44	—	13	13	18	18	+	†	†

* Parameter values in the CIMSIM and the field and laboratory observations upon which they were based have been presented earlier.* Temperatures at or below 20°C do not permit adult populations to develop because of the failure of eggs to hatch. Temperatures >34 or 35°C eliminate the possibility of adult populations because the aggregate survivals of all stages are insufficient.

† Not estimated at temperature extremes.

Because the human population grew at an approximate rate of 3.7% during the year, the density of people remained constant as DENSIM was set to dynamically model a corresponding larger area- population growth was accompanied by urban sprawl during the year. The initial seroprevalence of antibody was set to either 0%, 33%, or 67% in all age classes depending on the analysis being run. For each temperature, DENSIM was provided the appropriate temperature-specific values for daily gonotrophic development rate and weight of emerging females (Table 1) and on each day, the number of newly emerged females that were associated with a standing crop of 100 *Ae. aegypti* pupae per hectare (Table 2). By varying the number of people per hectare within DENSIM, we could evaluate the consequences of various ratios of pupae per person; e.g., telling DENSIM that the human population was 200 per hectare would correspond to 100 pupae per 200 people or 0.50 pupae per person.

The DENSIM was then iteratively run using these various ratios of *Ae. aegypti* and humans under conditions of monthly introductions of a single viremic individual and an initial seroprevalence of all age classes of antibody to dengue to be 0%, 33%, or 67%, our Monthly Introductions threshold. In a similar fashion, the second type of threshold, Single Introduction_{1, 2, 4, or 8}, was estimated, the number of pupae per person necessary to lead to an epidemic at least 50% of the time due to introduction(s) on calendar day 90 of 1, 2, 4, or 8 individual(s) at each the 3 levels of initial seroprevalence.

Iterative runs (approximately 14,000) were necessary because of the stochastic nature of incipient epidemics; typically, at the threshold ratio of pupae per person, only a portion (the goal being 50%) of the runs resulted in an epidemic increase >10% over the initial seroprevalence of antibody to dengue.

RESULTS

Gonotrophic development rate, adult size, and daily survivals. Given that the daily adult survival of females is independent of temperatures ranging between 22 and 32°C, a shorter gonotrophic cycle (Table 1) results in females at higher temperatures being expected to feed more often during their life times than at lower temperatures.⁸ For example, the results in Table 1 indicate that females at 32°C will attempt to take more than twice as many replete feeds as females at 24°C ($0.411/0.199 = 2.06$); a difference of only 4°C is significant—females at 30°C will take an average of approximately 30% more feeds than females at 26°C.

The size of adult *Ae. aegypti* females has been suggested to influence the proportion of females requiring two replete feeds on the first gonotrophic cycle; smaller females are therefore expected to be slightly more likely to transmit virus because of higher biting rates during the first cycle.^{6,8,9} Under larval rearing conditions where food is not limiting, CIMSIM generates estimates of smaller females at elevated

TABLE 2

The pupal development (PD_{temp}) period, expected daily number of newly-emerged females, and associated standing crop of females of all ages for a standing crop of 100 *Aedes aegypti* pupae as a function of temperature*

Temperature (°C)	PD_{temp} (days)	Number of adult females		Ratios of standing crops	
		New	Standing crop	Pupae/female	Females/pupa
22	4.06	10.22	87.7	1.14	0.88
24	3.33	12.46	106.9	0.94	1.07
26	2.66	15.61	133.9	0.75	1.34
28	2.04	20.37	174.8	0.57	1.75
30	1.46	28.44	244.0	0.41	2.44
32	0.92	45.20	387.9	0.26	3.88

* Methods of calculation are presented in the text and assume steady-state conditions and constant temperatures.

TABLE 3

Observed* and predicted† lengths of the pupal development (PD_{temp}) period

Temperature (°C)	\log_e (temperature)	PD_{temp} (days)	
		Predicted†	Observed*
15	2.7080	7.27	8.49
20	2.9957	4.86	3.11
22	3.0910	4.06	—
24	3.1780	3.33	—
25	3.2188	2.99	3.03
26	3.2581	2.66	—
27	3.2958	2.34	1.79
28	3.3322	2.04	—
30	3.4012	1.46	1.82
32	3.4657	0.92	—
34	3.5264	0.41	1.09

* Observed data from Rueda and others.¹⁵† Regression model used was $PD_{temp} = 29.97723 - 8.38467 \times \log_e(\text{temperature})$; $R^2 = 0.850$, standard errors and P values for the intercept and temperature coefficients were 5.6340 and $P = 0.006$, and 1.7593 and $P = 0.009$, respectively.

temperatures. However the projections of the CIMSiM of the weight of emerging females under field conditions (Table 1) does not indicate such a decrease; the weights are essentially uniform at temperatures ranging between 22 and 30°C. This observation is a result of lower larval competition at higher temperatures due to reduced survivals in the egg stage (Table 1).

Table 1 also presents the daily survival of adults (S_{adult}), eggs (S_{egg}), larvae (S_{larval}), and pupae (S_{pupal}), the possibility of egg hatch, and the possibility of adult populations under conditions of constant temperatures as indicated by CIMSiM. Parameter values in CIMSiM and the field and laboratory observations upon which they were based have been presented previously.⁸ Temperatures $\leq 20^\circ\text{C}$ do not permit adult populations to develop because of the failure of eggs to hatch. Temperatures > 34 or 35°C eliminate the possibility of adult populations because the aggregate survival of all stages combine to drive the population growth rate below replacement. Note at the temperatures considered herein, only egg survival varies significantly with temperatures ranging between 22 and 34°C . Adult survival begins to decrease at temperatures higher than 36°C and larval and pupal survivals begin to decrease as a function of elevated temperatures only at temperatures in excess of 39 or 40°C .

The relationship between standing crops of female adults and pupae as a function of temperature. The length of the pupal development (PD_{temp}) period influences the relationship between the standing crops of pupae and adults. Table 3 presents observed and predicted pupal development periods as a function of temperature based on data from Rueda and others.¹⁵ Note that the development period is the nominal approximately 2-days length only at 28°C , at 25°C it is approximately 50% longer, and at 32°C the period is only half of that at 28°C . The significance of this in terms of transmission can be seen in Table 2 where the number of newly-emerged females and associated standing crop of adult females arising from a standing crop of 100 *Ae. aegypti* pupae under steady-state equilibrium conditions as a function of temperature is presented. Here we see in addition to the reduction in gonotrophic development rate with temperature, another significant cause of increased transmission for a given ratio of pupae per person with elevated tempera-

TABLE 4

Estimated number of *Aedes aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of antibody to dengue during the course of a year resulting from 12 monthly viral introductions of a single viremic individual, the *Monthly Introduction* threshold*

Temperature (°C)	Transmission threshold by initial seroprevalence of antibody		
	0%	33%	67%
22	7.13	10.70	23.32
24	2.20	3.47	7.11
26	1.05	1.55	3.41
28	0.42	0.61	1.27
30	0.10	0.15	0.30
32	0.06	0.09	0.16

* In a series of simulations in the dengue simulation model, these values resulted in a 10% or greater increase in prevalence approximately 50% of the time.

tures—a given standing crop of pupae observed in the field will be associated with significantly higher female populations at elevated temperatures. The number of adult female *Ae. aegypti* associated with each pupa at 22°C is less than one; at 28°C , it has almost doubled to 1.75 females per pupa. Even a small temperature difference of only 4°C is important; for example, there are approximately 45% more females at 30°C than at 26°C for a given standing crop of pupae. Put another way, 100 pupae at 22°C are associated with a standing crop of approximately 90 females, whereas at 30°C the associated number is approximately 240.

The monthly introduction threshold. Under the conditions of our study, we have seen that temperature would be expected to influence probability of transmission in two ways: through 1) its role in determining the length of the gonotrophic cycle and thus the daily biting rate and through the impact of the pupal development period, and 2) its relationship to the ratio of number of pupae observed in a survey to the number of adult female *Ae. aegypti* mosquitoes in the same survey area. Another factor not documented in this study but incorporated in DENSiM is the influence of temperature on the extrinsic incubation period (EIP) of the virus in the female. At higher temperatures, infected females become infectious through viral dissemination at a significantly faster rate; thus, the probability of an infected female living long enough to become infectious goes up significantly with temperature. Specifically, the probability of surviving the incubation period is Sa^{EIP} . Using values for EIP from Focks and others⁶ for 22°C and 32°C of 16.67 and 8.33 days, respectively, the associated survival probabilities are 0.89^{16.67} or 0.143 at 22°C and 0.89^{8.33} or 0.379 at 32°C ; females incubating virus at the higher temperature are 2.64 times more likely to survive long enough to potentially infect human hosts.

Table 4 presents estimates of the Monthly Introduction thresholds incorporating the above-listed factors as a function of initial seroprevalence of dengue antibody and temperature. We have limited our range of estimates to temperatures between 22 and 32°C because field and laboratory observations serving as the basis of the parameter estimates in CIMSiM and DENSiM often did not include temperatures outside of this range. Initially, we will confine our comments to the thresholds at 26°C and 28°C , temperatures in the range of average annual temperatures experienced in many den-

TABLE 5

Comparison of observed numbers of *Aedes aegypti* pupae per person in various dengue-endemic or dengue-receptive locations with estimated transmission thresholds based on average summertime temperatures and an initial seroprevalence of 33%

Location	Temp (°C)*	Pupae per person†	Threshold‡	Ratio§	% Control¶
Reynosa, Mexico#	29.4	2.75	0.26	10.4	90
Mayaguez, Puerto Rico#	26.6	1.73	1.05	1.7	40
Trinidad (20 sites) ⁴	27.0	22.7**	0.86	26.4	96
El Progreso, Honduras ⁶	29.1	0.34	0.31	1.1	10
San Juan, Puerto Rico#	27.8	2.75	0.58	4.7	79
Bangkok, Thailand ^{5,9}	29.2	1.69	0.29	5.8	83

* Temp refers to average temperature during the months of June through August or December through February in locations above and below the equator, respectively.

† Pupae per person refers to the average number of *Ae. aegypti* pupae per person observed in a survey.

‡ Threshold refers to the estimated transmission threshold for 12 monthly introductions, assuming an initial seroprevalence of 33%.

§ Ratio is the ratio of observed pupae per person and the estimated temperature- and seroprevalence-specific threshold.

¶ % Control is the degree of reduction in pupae per person necessary to reduce observed field level to that of the threshold.

Unpublished studies conducted by one of the authors (DAF) in collaboration with others. Surveys in Puerto Rico and Mexico were limited and preliminary.

** Observed range = 1.4–63.4 pupae per person; the island-wide average is used for calculation.⁴

gue-endemic areas (Table 5). Our first observation is simply how very low these estimates are. Among dengue-naïve populations, the Monthly Introduction thresholds are estimated to be 1.05 and 0.42 *Ae. aegypti* pupae per person, respectively; given a level of herd immunity of 33%, the estimates rise to only 1.55 and 0.66, respectively. A comparison with observed numbers of pupae per person in various locations emphasizes this point (Table 5).

In light of the many factors increasing the probability of transmission at higher temperatures, it is not surprising that the threshold estimates for temperatures $>28^{\circ}\text{C}$ decrease quickly. Note that there is approximately a 4-fold decrease in the required number of pupae per person between 28 and 30°C but less than a 2-fold attenuation between 30 and 32°C ; this is the result of a flattening of the EIP-temperature relationship at higher temperatures in the 28– 32°C range (see Figure 3 in Focks and others⁶) and not to a reduction in daily adult survival at the more elevated temperatures. At temperatures $<26^{\circ}\text{C}$, the thresholds increase sharply and provide a partial explanation of the seasonal nature of transmission even in tropical locations where seasonal variation is not greater than a few degrees.

A final point of interest concerns the increase in thresholds with increasing initial seroprevalence of antibody to dengue virus. Independent of temperature, threshold values increase an average of 1.51-fold when the initial seroprevalence increases from 0% to 33%, 2.05-fold when going from 33% to 67%, and 3.15-fold when comparing an initial seroprevalence

0% with 67%. In light of the frequently high numbers of *Ae. aegypti* pupae per person observed (Table 5), it is not surprising that epidemic transmission is reported at even high levels of herd immunity. This would be especially true among infants and adolescents who typically have lower prevalences of antibody to dengue.

Multiple introduction thresholds. The patterns seen the among the transmission thresholds for the case of monthly introductions are also true for the single introductions of varying sizes (Tables 6 and 7). For each size of introduction, 1, 2, 4, or 8 individuals, there is a very uniform relationship between the threshold required for an initial seroprevalence of 0%, 33%, and 67%. Thresholds are an average of 150% higher for 33% than 0%, 206% higher for 67% than 33%, and 310% higher for 67% than in the case of an initial seroprevalence of 0%. The surprising result to us was the only slight decrease in threshold values with increasing numbers of viremic individuals introduced each year (Figure 1). It would appear that the probability of significant transmission is only slightly influenced by the size of the introduction. In practical terms, this means that any of our thresholds (Multiple or Monthly) can be used for risk assessment; this is especially true for the cases where the initial seroprevalence is $\leq 33\%$. In the case of an initial seroprevalence of 0%, the thresholds only decrease some 21% when comparing an introduction of 1 or 12 people; for initial seroprevalences of 33% and 67%, the reductions are 19% and 25%, respectively.

TABLE 6

Estimated number of *Aedes aegypti* pupae per person required to result in a 10% or greater increase in seroprevalence of antibody to dengue during the course of a year under conditions of a single viral introduction of 1 or 2 viremic individual(s) on day 90 of the year; the estimates for 2 individuals are in parentheses*

Temperature (°C)	Transmission thresholds by initial seroprevalence of antibody		
	0%	33%	67%
22	9.57 (9.16)	14.10 (12.83)	30.55 (29.15)
24	2.92 (2.68)	4.47 (4.21)	9.22 (8.68)
26	1.42 (1.23)	2.03 (1.98)	4.26 (4.01)
28	0.53 (0.48)	0.75 (0.72)	1.69 (1.38)
30	0.13 (0.12)	0.19 (0.18)	0.38 (0.35)
32	0.07 (0.07)	0.10 (0.10)	0.26 (0.18)

* In a series of simulations in the dengue simulation model, these values resulted in a 10% or greater increase in prevalence approximately 50% of the time.

TABLE 7

Estimated number of *Aedes aegypti* pupae per person required to result in a 10% or greater increase in seroprevalence of antibody to dengue during the course of a year under conditions of a single viral introduction of 4 or 8 viremic individuals on day 90 of the year; the estimates for 2 individuals are in parentheses*

Temperature (°C)	Transmission threshold by initial seroprevalence of antibody		
	0%	33%	67%
22	8.02 (7.13)	11.66 (10.69)	24.66 (22.11)
24	2.52 (2.20)	3.69 (3.27)	7.76 (7.02)
26	1.09 (1.08)	1.80 (1.57)	3.79 (3.24)
28	0.47 (0.41)	0.63 (0.62)	1.33 (1.27)
30	0.11 (0.09)	0.18 (0.15)	0.33 (0.31)
32	0.06 (0.06)	0.09 (0.09)	0.18 (0.16)

* In a series of simulations in the dengue simulation model, these values resulted in a 10% or greater increase in prevalence approximately 50% of the time.

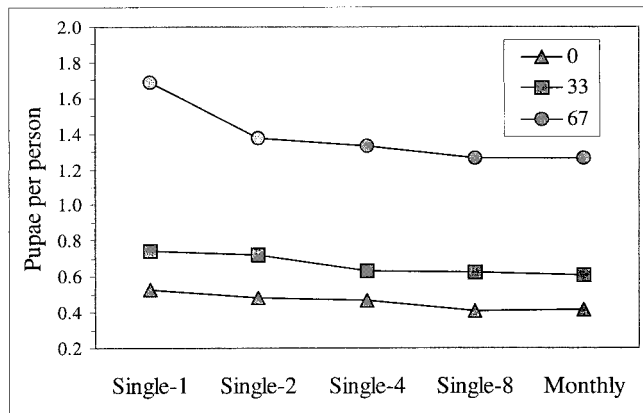


FIGURE 1. Transmission thresholds at 28°C for each type of introduction by initial seroprevalence of antibody.

DISCUSSION

In the present work, stochastic models were used to estimate transmission thresholds for dengue. The estimates presented, at least for ambient air temperatures ranging between 26 and 30°C, are consistent with the only field estimate available: <0.25 *Ae. aegypti* pupae per person in Honduras, and with a theoretical estimate made by Newton and Reiter for Puerto Rico.^{6,14} Substantial validation will await the results of a 5-year National Institutes of Health-funded study entitled *Entomological Assumptions of Dengue Control* being conducted in Iquitos, Peru. The study is developing spatially based time series of entomologic and serologic parameters based on surveys of several thousand households and seroconversion rates among more than a thousand children. Our intention is that these estimates will be of value in directing control operations and influencing policies pertaining to dengue. In our view and in the view of others, much of what is currently accepted or recommended regarding source reduction for control is questionable and in need of modification.^{3,4} Our concluding discussion focuses on some aspects of dengue control that could be improved using transmission thresholds and pupal and demographic surveys.

In light of estimates of the actual density of *Ae. aegypti* per person seen in various dengue-prone areas (Table 5), the results of this analysis are not particularly encouraging from the perspective of controlling dengue through sustained suppression of the vector via source reduction. They do provide a basis for evaluating the probability of success of source reduction efforts. The ratio of *Ae. aegypti* pupae to human density has been observed in limited field studies to range between 0.34 and >60 in 25 sites in dengue-endemic or dengue-susceptible areas in the Caribbean, Central America, and Southeast Asia. If, for purposes of illustration, we assume an initial seroprevalence of 33%, in Puerto Rico, Honduras, and Bangkok, Thailand, the degree of suppression required to essentially eliminate the possibility of summertime transmission was estimated to range between 10% and 83%; in Mexico and Trinidad, reductions of $>90\%$ would be required. A clearer picture of the actual magnitude of the reductions required to eliminate the threat of transmission is provided by the ratio of the observed standing crop of *Ae. aegypti* pupae per person and the threshold. For example, in

a site in Mayaguez, Puerto Rico, the ratio of observed and threshold was 1.7, meaning that approximately 7 of every 17 breeding containers would have to be eliminated. For Reynosa, Mexico, with a ratio of approximately 10, 9 of every 10 containers would have to be eliminated. For sites in Trinidad with ratios averaging approximately 25, the elimination of 24 of every 25 would be required. With the exceptions of Cuba and Singapore, no published reports of sustained source reduction efforts have achieved anything near these levels of reductions in breeding containers. Promoting source reduction as a viable and practical means of dengue control, but without consideration of the degree of elimination required, is reminiscent of an earlier era when aerosol sprays for adult mosquitoes were recommended with insufficient information on efficacy.

The underlying notion of targeted source reduction is one of selectively attacking the most important types of containers. Field observations suggest the rationale is sound in that containers vary significantly in their production of *Ae. aegypti*. Southwood and others reported for a temple area in Bangkok an approximately 23-fold difference in the most and the least productive types of container;⁵ a 6-fold difference was seen in Honduras.⁶ The actual epidemiologic significance of any particular type of container, say discarded tires, is a function of the average standing crop of pupae found in that type and the abundance of that container. Table 8 is an example of how transmission thresholds and the pupal and demographic survey could provide guidance to a targeted source reduction effort. The estimate of the transmission threshold provides an overall target, an upper bound on the number of pupae per person for the environment that insures that viral introductions would result in very little or no transmission. The survey permits estimating the contribution of each type of container and allows, using nothing more than a spreadsheet, conducting what-if analyses of various strategies designed to selectively attack different types of containers at various rates of elimination based on their epidemiologic importance and how amenable they are to elimination and/or control.

Our example is based on surveys conducted during June 1995 in urban areas of central St. George County in northern Trinidad.⁴ Based on average temperatures for this period (27.8°C) and assuming a seroprevalence rate of 33%, the estimate of the transmission threshold is approximately 0.71 pupae per person (interpolation of Table 4). The surveys estimated human densities to be approximately 160 per hectare and provided data on the 9 major types of breeding containers, their abundance, and average standing crop of *Ae. aegypti* pupae (Table 8). In this environment, there was an average of approximately 98 water-filled containers and 209 pupae per hectare; the number of pupae per person was 1.31 or 184% of the threshold. Numerically, the two most common types were indoor containers, the flower vase and water storage drum. Notice, however, that because these types differed significantly in productivity, the epidemiologic significance, based on their contribution to the number of pupae per hectare or per person, of the indoor drum is some 40-times more important. Dividing the estimate of pupae per person for each type by the threshold of 0.71 yields an estimate of what proportion of the threshold is contributed by each; this indicates the vases contribute $<2\%$ of the thresh-

TABLE 8

An example of survey results from urban sites in St. George County of Trinidad conducted during June 1995 incorporating a transmission threshold estimate of 0.71 pupae per person^{4*}

Container type	Containers per ha	Pupae per container	Pupae per ha	Pupae per person	Portion of threshold	Relative importance
Saucer	3.9	0.20	0.8	0.005	0.007	0.004
Tire	0.8	1.00	0.8	0.005	0.007	0.004
Small miscellaneous	1.2	1.10	1.3	0.008	0.012	0.006
Indoor vase	40.0	0.05	2.0	0.013	0.018	0.010
Tank	9.5	0.40	3.8	0.024	0.034	0.018
Bucket	1.1	10.90	12.0	0.075	0.106	0.057
Tub	13.5	3.80	51.3	0.321	0.452	0.245
Outdoor drum	8.3	6.70	55.6	0.348	0.490	0.266
Indoor drum	19.4	4.20	81.5	0.509	0.719	0.390
Totals	97.7	—	209.1	1.307	1.844	1.000

* The threshold estimate is based on interpolating values in Table 4 using an average June temperature of 27.7°C and an overall seroprevalence of 33%. Pupae per hectare (ha) is the product of Containers per ha and Pupae per container. Pupae per person is the ratio of Pupae per ha and the average human density of 160 per ha. Portion of threshold is the ratio of Pupae per person and the threshold estimate. Relative importance is the ratio of Pupae per person for each container type and the total number of pupae per person = 1.307. Putting data like these and their relationships into a spreadsheet permits what-if analyses of the anticipated impact of various targeted source reduction strategies.

old whereas the indoor drum accounts for >70%. Obviously, if eradication is not in mind, targeting the more important types based on this logic would suggest a focus on indoor and outdoor drums and perhaps the tubs. If Table 8 is put into a spreadsheet, evaluating various targeted strategies becomes easy. We see that with an overall reduction of approximately 50% of all containers, the control or elimination of about 50 of the 100 containers would result in the number of pupae per person being about 92% of the threshold. We also can see that a targeted approach that eliminated about 55% of the 3 most important types, the 2 types of drums and tubs, would put the population at about 93% of threshold, and would require the control of only about 23 containers per hectare. This approach would also allow solving for the required reductions by type given some types were uncontrollable by virtue of their location, ownership, and use.

We are developing software with funding from the Strategic Environmental Research and Development Program of the U.S. Departments of Defense and Energy, and Environmental Protection Agency (EPA) for the Windows 95/98 and NT® platforms that provides spatially based risk assessment and guidance for the control of dengue. Briefly, the software provides on-line instruction on how to conduct a spatially-based survey of the number of pupae per person, and then provides a map indicating regions where this is sufficient to permit transmission. The software also provides aid for source reduction control efforts by identifying the major types of breeding containers and their contributions to the transmission threshold as presented earlier. The software permits the acquisition of base maps using virtually any source of data: Computer-Aided Design drawings, Geographic Information System files such as Tiger and Digital DEMs, and paper maps and aerial photos via scanner or digital camera. It geographically locates and scales the base map using the Global Positioning System and/or the geo-referencing functionality of the software. Finally, it evaluates interactively and graphically the impact of various levels of targeted source reduction. When completed, this will be made available without cost to anyone probably through the Web and CD-ROM.

Acknowledgments: The simulations were conducted by Michael Ming Ho (University of Florida Science Training Program) and Mo-

han Punja (summer volunteer, U.S. Department of Agriculture, Agricultural Research Service).

Financial support: The research reported in this paper was supported in part by funds from Pollution Prevention Project No. 1053, Strategic Environmental Research and Development Program (SERDP) and EPA-USDA Interagency Agreement No. DW12937600-01-0.

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